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The *vanB* gene of vancomycin-resistant *Enterococcus faecalis* V583 is structurally related to genes encoding D-Ala:D-Ala ligases and glycopeptide-resistance proteins VanA and VanC*

(D-alanine:D-alanine ligase; cell wall; peptidoglycan synthesis)

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SUMMARY

We report the cloning and sequencing of a 632-bp amplified fragment internal to the *vanB* gene of vancomycin-resistant (Vm^R) *Enterococcus (En.) faecalis* V583. The DNA fragment hybridized to Vm^R strains of *En. faecium* and *En. faecalis*, but not to their susceptible derivatives.

Glycopeptide antibiotics vancomycin (Vm) and teicoplanin (Te) bind to the C-terminal D-Ala residues of peptidoglycan precursors blocking their incorporation into the bacterial cell wall (Reynolds, 1989). These residues are incorporated into cell wall precursors as a dipeptide synthesized by D-Ala:D-Ala ligases (Ddl) (Walsh, 1989). The VanA ligase synthesizes the depsipeptide D-Ala-D-Lac which substitutes for D-Ala-D-Ala leading to synthesis of precursors which bind Vm with reduced affinity (Bugg et al., 1991; Handwerger et al., 1992; Messer and Reynolds, 1992).

Glycopeptide resistance in enterococci is heterogeneous (Dutka-Malen et al., 1990). Resistance proteins

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  L  F  E  L  S  G  I  P  Y  V  G  C  D  I  Q  S  S  A  A  C..20
TCTGTTTGAATTGTCTGGTATCCCCTATGTAGGCTGCGATATTCAAAGCTCCGCAGCTTG  60
  M  D  K  S  L  A  Y  I  L  T  K  N  A  G  I  A  V  P  E  F  40
CATGGACAAATCACTGGCCTACATTCTTACAAAAAATGCGGGCATCGCCGTCCCCGAATT 120
  Q  M  I  E  K  G  D  K  P  E  A  R  T  L  T  Y  P  V  F  V  60
TCAAATGATTGAAAAAGGTGACAAACCGGAGGCGAGGACGCTTACCTACCCTGTCTTTGT 180
  K  P  A  R  S  G  S  S  F  G  V  T  K  V  N  S  T  E  E  L  80
GAAGCCGGCACGGTCAGGTTCGTCCTTTGGCGTAACCAAAGTAAACAGTACGGAAGAACT 240
  N  A  A  I  E  A  A  G  Q  Y  D  G  K  I  L  I  E  Q  A  I 100
AAACGCTGCGATAGAAGCAGCAGGACAATATGATGGAAAAATCTTAATTGAGCAAGCGAT 300
  S  G  C  E  V  G  C  A  V  M  G  N  E  D  D  L  I  V  G  E 120
TTCGGGCTGTGAGGTCGGCTGCGCGGTCATGGGAAACGAGGATGATTTGATTGTCGGCGA 360
  V  D  G  I  R  L  S  H  G  I  F  R  I  H  Q  E  N  E  P  E 140
AGTGGATCAAATCCGGTTGAGCCACGGTATCTTCCGCATCCATCAGGAAAACGAGCCGGA 42J
  K  G  S  E  N  A  M  I  I  V  P  A  D  I  P  V  E  E  R  N 160
AAAAGGCTCAGAGAATGCGATGATTATCGTTCCAGCAGACATTCCGGTCGAGGAACGAAA 480
  R  V  Q  E  T  A  K  K  V  Y  R  V  L  G  C  R  G  L  A  R 180
TCGGGTGCAAGAAACGGCAAAGAAAGTATATCGGGTGCTTGGATGCAGAGGCCTTGCTCG 540
  V  D  L  F  L  Q  E  D  G  G  I  V  L  N  E  V             196
TGTTGATCTTTTTTTGCAGGAGGATGGCGGCATCGTTCTAAACGAGGTC             589
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Fig. 1. Nucleotide and corresponding aa sequence of the PCR fragment internal to the *vanB* gene. The nt sequence of both strands was determined from a pUC18 insert by the dideoxy-chain-termination method (Sanger et al., 1977) using T7 DNA polymerase. The sequences complementary to oligos V1 and V2 (Dutka-Malen et al., 1992) are not shown. Additional experiments were carried out to eliminate the possibility of nt misincorporation by the *Taq* DNA polymerase. GenBank accession No. is L06138.

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* On request, the authors will supply experimental evidence for the conclusions reached in this brief note.

Abbreviations: aa, amino acid(s); bp, base pair(s); D-Ala, D-alanine(s); DdlA and DdlB, D-Ala:D-Ala ligases of *E. coli*; D-Lac, D-lactate; *E.*, *Escherichia*; *En.*, *Enterococcus*; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); oligo, oligodeoxyribonucleotide; PCR, polymerase chain reaction; ^{R}, resistant; ^{S}, sensitive; Te, teicoplanin; VanA, *En. faecium* Vm-resistance-conferring protein; VanB, *En. faecalis* Vm-resistance-conferring protein; VanC, *En. gallinarum* Vm-resistance-conferring protein; *vanB*, gene encoding VanB; Vm, vancomycin.

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VanB  LFELSGIPYV GCDIQSSAAC MDKSLAYILT ENAGIAVPEF QMIERGDKP- --------EA RTLTYPVFVK PARSGSSFGV TKVNSTEELN AAIEAAGQYD GKILIEQAIS 101
VanA  LFELSGIPFV GCDIQSSAIC MDKSLTYIVA KNAGIATPAF WVINKDDRP- --------VA ATPTYPVFVK PARSGSSFGV KKVNSADELD YAIESARQYD SKILIEQAVS 101
VanC  LLELMNLPYV GCHVAASALC MNKWLLHQLA DTMGIASAPT LLLSRYE.D- --PATIDRFI QDHGFPIFIK PNEAGSSKGI TKVTDKTALQ SALTTAFAYG STVLIQKAIA 107
DdlA  MLRVANLPFV GSDVLASAAC MDKDVTKRLL RDAGLNIAPF ITLTRANRHN ISFAE---VE SKLGLPLFVK PANQGSSVGV SKVTSEEQYA TAVALAFEFD HKVIVEQGIK 107
DdlB  MLELMGLPYT GSGVMASALS MDKLRSKLLW QGAGLPVAPW VALTRAEFEK GLSDKQLAEI SALGLPVIVK PSREGSSVGM SKVVAENALQ DALRLAFQHD EEVLIEKWLS 110
      CC  · CI   IC C  II C I I     C     IC       C C                      ICCCI I   III IC  II        IC  I       CCC  CC

VanB  GCEVGCAVMG NEDDLIVGEV DQIRLSHGIF RIHQENEPEK GSENAMIIVP ADIPVEERNR VQETAKKVYR VLGCRGLARV DLFLQEDGGI VLNEV 196
VanA  GCEVGCAVLG NSAALVVGEV DQIRLQYGIF RIHQEVEPEK GSENAVITVP ADLSAEERGR IQETAKKIYK ALGCRGLARV DMFLQDNGRI VLNEV 196
VanC  GIEIGCGILG NE-QLTIGAC DAISLVDGFF DFEEKYQLIS ----ATITVP APLPLALESQ IKEQAQLLYR NLGLTGLARI DFFVTNQGAI YLNEI 197
DdlA  GREIECAVLG NDNP----QA STCGEIVLTS DFYAYDTKYI DEDGAKVVVP AAIAPEINDK IRAIAVQAYQ TLGCAGMARV DVFLTPENEV VINEI 198
DdlB  GPEFTVAILG EEIL------ -PSIRIQPSG TFYDYEAKYL SDETQYFC-P AGLEASQEAN LQALVLKAWT TLGCKGWGRI DVMLDSDGQP YLLEA 198
      I IC  CCCI                                    I I C       C          II  I CIC ICCC    C  C I
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Fig. 2. Alignment of the deduced partial aa sequence of VanB and of the corresponding regions of VanA, VanC, DdlA and DdlB (Dutka-Malen et al, 1992). Identical aa (I) and conservative substitutions (C) in the five sequences are indicated below the alignment. For classification as conservative substitutions, the aa were grouped as follows: RK, LFPMVI, STQNC, AGW, H, ED and Y.

VanA and VanC display 28 to 38% aa identity with Ddl of *E. coli* (Dutka-Malen et al.,1992). The structural genes for VanA and VanC do not hybridize with DNA of enterococci that become resistant to Vm only after induction (VanB phenotype) (Dutka-Malen et al, 1990; Leclercq et al., 1992).

Oligos V1 and V2 allow PCR amplification of fragments internal to genes encoding VanA, VanC, and Ddl (Dutka-Malen et al., 1992). These oligos prime the amplification of ca. 600-bp fragments from *En. faecalis* V583 and *En. faecium* D366 which display the VanB phenotype (Sahm et al., 1989; Gutmann et al., 1992). The fragments from strain V583 were cloned into pUC18 (Norrander et al., 1983) and the insert of a recombinant plasmid was sequenced (Fig. 1). The deduced aa sequence of the insert was similar to a portion of VanA (77% aa identity), of VanC (37%) and of Ddl of *E. coli* (30 and 32%) (Fig. 2). In Southern hybridization, the cloned fragment hybridized with a 3.3-kb *Hin*dIII-*Kpn*I fragment of *En. faecalis* V583 and a 7.5-kb *Hin*dIII-*Kpn*I fragment of *En. faecium* D366 (data not shown). The probe did not hybridize to DNA from either Vm^S derivatives of these strains or Vm^S *En. faecalis* and *En. faecium* reference strains. These results suggest that the cloned PCR product corresponds to an internal fragment of a resistance-conferring gene acquired by the Vm^R strains. This gene encoded a Ddl-related enzyme, designated VanB, which could be involved in the synthesis of a substitute for D-Ala-D-Ala. This hypothesis is consistent with preliminary characterization of peptidoglycan precursors from *En. faecium* D366 (Billot-Klein et al., 1992).

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